

(e.g., a collimating lens) and a detector (e.g., a CCD or CMOS camera) are used to collect data from the reporters and the first and second calibration standards.

**[0031]** When the sample **220** is applied to the diffusion strip of the lateral-flow chromatographic assay cassette **205**, the liquid in the sample carries the analyte of interest through the diffusion strip in flow direction **225** into the analysis zone **230** where it can be captured by the capture ligand line **240**. The first and second calibration standard lines **250** and **254** are selected to provide a detectable signal that correlate to non-zero concentration values of the analyte of interest. For example, the first and second calibration standard lines **250** and **254** may include an amount of the analyte of interest or another material pre-bound to the diffusion strip of the lateral-flow chromatographic assay cassette **205**. The reporter **260** may be a diffusible material that can bind to the capture ligand line **250** and the first and second calibration standards **250** and **254** in an amount proportional to the amount of bound ligand is present in each line. In response to illumination by the light source, the reporter **260-264** bound to each of lines **250-254** provides a signal that can be used to calculate a calibration curves and, in turn, determine the concentration of the analyte of interest in the sample **220**.

**[0032]** FIG. 3A illustrates an apparatus **300** that can be used to test the relationship between illumination angle, detection angle, and fluorescent signal. The apparatus **300** includes an illumination light source **320**, a detector device **340**, means **330** for transmitting an illuminating light from the illumination source **320** to the lateral-flow chromatographic assay cassette **100**, means **350** for transmitting a signal from the lateral-flow chromatographic assay cassette **100** to the detector device **340**, and an adjustable variable angle stage **310** configured for adjusting an angle of the lateral-flow chromatographic assay cassette **100** in relation to an illuminating light source and a detector device.

**[0033]** In the illustrated embodiment, the means **330** for transmitting an illuminating light from the illumination source to the lateral-flow chromatographic assay cassette **100** includes an optical fiber. In other embodiment, the means **330** for transmitting an illuminating light from the illumination source to the lateral-flow chromatographic assay cassette **100** may include at least one of a light pipe or one or more lenses. Likewise, in the illustrated embodiment, the means **350** for transmitting a signal from the lateral-flow chromatographic assay cassette **100** to the detector device **340** includes an optical fiber. Other options include at least one of a light pipe or one or more lenses. In the illustrated embodiment, optical fibers **330** and **350** are supported by supports **335** and **355**, respectively.

**[0034]** The illuminating light **330** and the and the detector device (or the means **350** for transmitting a signal from the lateral-flow chromatographic assay cassette **100** to the detector device **340**) are positioned to illuminate an analysis region **360** of the lateral-flow chromatographic assay cassette **100** and the adjustable variable angle stage **310** is adjustable such that an angle of illumination and an angle of reflection are adjusted in relation to the lateral-flow chromatographic assay cassette **100** so as to optimize an elastic light scattering signal from the lateral-flow chromatographic assay cassette **100**.

**[0035]** FIG. 3B illustrates the principle of adjusting the angle of the illuminating light **330** and the detector device (or the means **350** for transmitting a signal from the lateral-flow chromatographic assay cassette **100** to the detector device **340**) relative to the analysis region **360** of the lateral-flow

chromatographic assay cassette **100** in greater detail. In the embodiment illustrated in FIG. 3B, there is an angle  $\alpha$  between the illuminating light **330** and the detector device (or the means **350** for transmitting a signal from the lateral-flow chromatographic assay cassette **100** to the detector device **340**). By adjusting the angle  $\alpha$  with the adjustable variable angle stage **310** (FIG. 3A), the elastic light scattering signal from the analysis region **360** of the lateral-flow chromatographic assay cassette **100** can be optimized.

**[0036]** Referring now to FIG. 4, another embodiment of a sample holder **400** is illustrated. The sample holder **400** may, for example, be coupled directly to a light source and a detector device. The sample holder **410** includes a cassette port **410** that is configured such that a lateral flow immunoassay cassette **100** can be inserted into the sample holder **400**. In addition, the cassette port **410** of the sample holder **400** includes an adjustable variable angle device **430** (e.g., a rotatable dial) that allows angle of the cassette **100** to be adjusted relative to a light source and a detector device. By selectively modifying these angles, the lower detection limit of the assay can be extended, the signal to noise ratio can be improved, etc. In one embodiment, the device can be adjusted manually in order to choose an angle that optimizes detection limit, signal to noise, and the like. In another embodiment, the device can be coupled to a mechanical means, such as a servo motor or a gel-damped spring device that can allow the device to automatically sample a number of angles while a detector device collects data from the lateral-flow chromatographic immunoassay cassette **100**.

**[0037]** In addition, the cassette port **410** of the sample holder **400** includes a sealing gasket **420** disposed around the cassette port **410** that can seal the cassette port **410** when an assay cassette **100** is inserted therein so that ambient light does not leak into the sample holder **400**. For example, if ambient light leaks into the sample holder **400**, it could skew results. In addition, the cassette port **410** may include a spring-loaded flap (not shown) or similar means that can seal ambient light out of the sample holder **400** even when no cassette **100** is inserted into the cassette port **410**.

**[0038]** The inventors have conducted preliminary experiments using the apparatus **300** illustrated in FIG. 3A and a lateral-flow chromatographic immunoassay cassette that is set up to detect thyroid-stimulating hormone ("TSH"). This assay uses ~50 microliters of capillary blood, which is applied at the sample application zone to a nitrocellulose membrane housed in the cassette. At the membrane origin are mobile phase anti-TSH antibodies labeled with colloidal gold. A diluent is deposited on the blood spot and the blood travels the length of the membrane past a test line (which consists of a solid phase capture antibody) and a control line. This is similar to the format of a home pregnancy test. The data shown in FIG. 6 illustrate that by varying the angles of incident and reflected light it is possible to detect the presence of colloidal gold (e.g., signal) at the test line with varying degrees of sensitivity. By optimizing these angles, it may be possible to extend the lower limit of detection of the assay. That is, make it more sensitive.

**[0039]** For example, a standard reference range for TSH for adults is between 0.4 and 5.0  $\mu\text{IU/mL}$ . The therapeutic target range TSH level for patients on treatment ranges between 0.3 to 3.0  $\mu\text{IU/L}$ . TSH levels for children normally start out much higher with age-related reference limits starting from about 1.3 to 19  $\mu\text{IU/mL}$  for normal-term infants at birth, dropping to